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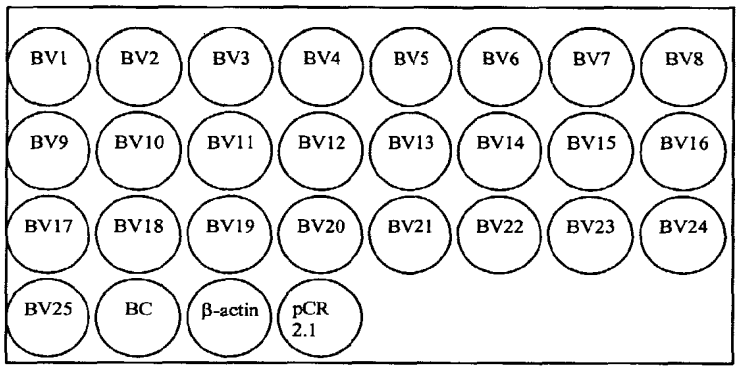
(54) **Title:** METHOD OF DETECTING T-CELL PROLIFERATION FOR DIAGNOSIS OF DISEASES BY GENE ARRAY



(57) **Abstract:** The present invention is directed to a method of detecting over-expression of certainT-cell receptor V genes in a sample. The method uses a T-cell receptor gene arraycontaining a substrate with a plurality of positions, each position having an immobilizednucleic acid complementary to a fragment of various families of the human T-cellreceptor V genes. Nucleic acids are extracted from a sample and labelled, then contactedwith the T-cell receptor gene array to allow complementary sequences to hybridize.After the unhybridized nucleic acids are removed, the one or more positions that haveelevated signals are identified and the over-expressed T-cell receptor V genes aredetected. The present invention is also directed to a kit comprising the T-cell receptorgene array for detecting over-expression of certain T-cell receptor V genes in a sample.The present invention is useful in diagnosing autoimmune diseases such as multiplesclerosis, rheumatoid arthritis, insulin-dependent diabetes mellitus, type I diabetes,inflammatory bowel disease, psoriasis, system lupus erythematosus, and Crohn'sdisease or T cell associated malignancies such as T cell leukemia and T cell lymphoma.

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METHOD OF DETECTING T-CELL PROLIFERATION FOR DIAGNOSIS OF DISEASES BY GENE ARRAY

TECHNICAL FIELD

The present invention generally relates to medical diagnosis and disease monitoring. More specifically, the present invention relates to a method of detecting a pathological state of humans by detecting over-expression of certain T-cell receptor V genes characteristic of clonal activation and expansion.

BACKGROUND OF THE INVENTION

The receptors recognizing antigens at the surface of mature T lymphocytes (hereafter designated T-cell antigen receptors or TCRs) possess a structure having a certain similarity with those of immunoglobulins. Therefore, they contain heterodimeric structures containing α and β glycoprotein chains or γ and δ glycoprotein chains.

The directory of T-cell receptors must be able to address the immense diversity of antigenic determinants. This is obtained by genetic recombination of different discontinuous segments of genes which code for the different structural regions of T-cell receptors. Thus, the genes contain V segments (variable segments), optionally D segments (diversity segments), J segments (junction segments) and C segments (constant segments). During the differentiation of T-cells, specific genes are created by recombination of V, D and J segments for the β and δ loci and V and J segments for the α and β loci. These specific combinations as well as the pairing of two chains create the combinational diversity. This diversity is highly amplified by two supplementary mechanisms, namely the imprecise recombination of V-D-J or V-J segments and the addition of nucleotides corresponding to the N region (Davis et al., *Nature* 334:395 (1988)). The genes encoding the T-cell receptor (TCR) α and β chains are produced by the combination of the Vα, Jα and Cα or Vβ, Jβ, Dβ, and Cβ segments respectively.

More than 70 Vα and Vβ gene segments have been molecularly characterized and are classified into 29 and 25 subfamilies, respectively, on the basis of sequence similarity in their coding regions. These distinct levels of TCR diversity allow the generation of a large T cell repertoire able to face the large diversity of short peptide bound to the MHC molecules. Hypervariable complementary determining region-3 (CDR3)-like loops encoded by V(D)J junctions are thought to interact directly with the antigenic peptide. The characterization of TCR polypeptides is a way to precisely analyze T cell responses. In this respect, the CDR3

sequence defines a unique TCR clonotype. It is predicted that antigen-driven T cell expansion *in vivo* would lead to the discovery of recurrent TCR transcripts and the finding of multiple isolates of a single clonotype to indicate clonal expansion.

Clonal activation and expansion of pathogenic T-cells is the immunological hallmark of various human autoimmune diseases, including rheumatoid arthritis and multiple sclerosis. It is also seen in other human pathological conditions, such as T-cell leukemia and lymphoma. Currently, it is considered extremely difficult to identify the clonal activation and expansion of T-cells in the above-mentioned diseases due to lack of technical means. In particular, autoimmune T-cells in several autoimmune pathological conditions represent only a minor population of all circulating T-cells, making the detection almost impossible.

Some methods and/or kits are currently available for detecting an autoimmune disease such as rheumatoid arthritis and multiple sclerosis. For example, U.S. Patent No. 5,445,940 discloses that a subset of human patients having an autoimmune disease were detected using monoclonal antibodies, fragments, and derivatives thereof reactive with an epitope of the T-cell receptor alpha chain variable region, Vα 12.1, on human T lymphocytes. The monoclonal antibodies were reactive with approximately 2% of $CD4^+$ T lymphocytes and with approximately 5% of $CD8^+$ T lymphocytes in peripheral blood cells in normal individuals and defined a subset of individuals afflicted with an autoimmune disease, especially rheumatoid arthritis, that exhibit increased expression of the Vα gene on $CD8^+$ peripheral blood T lymphocytes when compared to normal individuals.

Another example is the usage of B- and T-cell clonality assay kits in the early diagnosis and differential diagnosis for multiple sclerosis and other neurological diseases as disclosed in Qin (WO 99/15696). Qin discloses that the B-cell clonal expansion is present in the majority of multiple sclerosis patients, and that detection of B-cell clonal expansion could be used for diagnosing the disease.

Although all T-cells express a complete set of T-cell receptors families, *in vivo* activation and expansion of pathogenic T-cells of limited clonal lineage results in over-expression of certain T-cell receptor variable (V) gene families characteristic of pathogenic T-cells. Clonal expansion of pathogenic T-cells can be detected by identifying over-expression of only certain V genes in patient's blood or other body fluid specimens. The identification of the over-expression of certain V genes serves the purposes of diagnosis and disease monitoring since pathogenic T-cells are associated with the clinical course and pathology of respective diseases.

Rezvang, *et al.*, (*Blood*, 44:1063-1069 (1999)) report TCRBV (T-cell receptor B variable) gene usage and CDR 3 size distribution using reverse transcription PCR. Farace, *et al.*, (*J. Immunology*, 153:4281 (1994)) report analyzing TCR Vα and Vβ gene-segment by PCR using a panel of V gene-segment subfamily-specific oligonucleotide primers (Vα 1-29/Vβ 1-24). To use traditional PCR technology to analyze TCR, a set of primers specific for the V genes is synthesized and used for PCR detection. Each sample must be analyzed with different pairs of primers from the TCR Vα and TCR Vβ subfamilies. As each pair of primers has different efficiency and different requirement for PCR conditions (e.g., annealing temperature), the traditional PCR method is not suitable for quantitative detection of T-cell receptor V genes in the blood and tissue specimens where V genes of clonally expanded pathogenic T-cell populations are often obscured among those of unrelated T-cells. Furthermore, it is highly labor-intensive to run multiple PCR experiments for one sample and with the sample being prone to contamination due to high sensitivity of PCR. Therefore, there is clearly a need for an assay with high specificity and sensitivity to quantitatively and efficiently detect over-expression of certain T-cell receptor V genes.

SUMMARY OF INVENTION

The present invention is directed to a method of detecting over-expression of certain T-cell receptor V genes in a sample. The method uses a T-cell receptor gene array containing a substrate with a plurality of positions, each position having an immobilized nucleic acid complementary to a fragment of various families of the human T-cell receptor V genes. Nucleic acids are first extracted from a sample such as blood or other body fluid, and then labelled with a signalling molecule. The labelled nucleic acids are contacted with the T-cell receptor gene array under conditions that allow complementary sequences to hybridise. After the unhybridized nucleic acids are removed, the one or more positions that have elevated signals compared with other position are identified and the over-expressed T-cell receptor V genes are detected.

The present invention is useful in diagnosing autoimmune diseases or T cell associated malignancies. Autoimmune diseases suitable for the present invention are multiple sclerosis, rheumatoid arthritis, insulin-dependent diabetes mellitus, type I diabetes, inflammatory bowel disease, psoriasis, system lupus erythematosus, and Crohn's disease. T cell associated malignancies suitable for the present invention are T cell leukemia or T cell lymphoma.

The present invention is also directed to a kit for detecting over-expression of certain T-cell receptor V genes in a sample comprising a T-cell receptor gene array, said array containing a substrate comprising a plurality of positions, each position having an immobilized nucleic acid complementary to a fragment of various families of the human T-cell receptor V genes.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 depicts the format of the TCR gene array membrane design.

Fig. 2 shows the detection of TCRBV genes of SEB stimulated normal peripheral blood lymphocytes.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to a method for detecting over-expression of certain T-cell receptor V genes characteristic of clonal activation and expansion, in samples such as patient specimens and cell cultures. The method uses a T-cell receptor gene array containing a substrate with plurality of positions, each position having an immobilized nucleic acid complementary to a fragment of various families of the human T-cell receptor V genes. The T-cell receptor gene array is used to quantify various TCR V genes in a sample. An object of the present invention is to provide an assay system and a method that can distinguish between various T-cell receptor V genes.

In the method to detect T cell clonal expansion and TCR V gene distribution pattern in a given sample, e.g. cell culture, blood, tissue or any body fluid, RNAs are extracted from the sample and mRNAs/tRNAs are then prepared. The resulting mRNAs/tRNAs are subsequently reversed transcribed to cDNAs and then the cDNAs are labeled with signal generating agents such as radioactive isotope, biotin, fluorescence or a chemiluminescent agent. The labeled cDNAs are then hybridized with the T-cell receptor gene array under conditions that allow complementary sequences to hybridize. The non-hybridized nucleic acids are removed. The array is then analyzed to detect one or more positions that have elevated signals compared with other positions; the positions that have elevated signals refer to the over-expressed T-cell receptor V genes.

Unlike other methods for TCR repertoire, such as conventional PCR, immunoassay and southern blot analysis, by which only the one TCR gene can be analyzed in one assay, the present invention utilizes gene-based TCR array, which can analyze the expression of multiple or even a complete set of TCR V gene in a single hybridization assay. For example, more than 25 TCR V genes involved in an individual pathway can be assessed in one experiment. The

experimental procedures for performing gene-based TCR array are simpler and faster than conventional methods since they do not require multiple RNA gel electrophoresis and laborious transferring of materials. In addition, TCR gene results are shown on the same array membrane, which are easy to interpret.

Preparation of Immobilized DNAs.

The T-cell receptor gene array of the present invention contains a substrate with a plurality of defined positions. Each defined position has a specific immobilized nucleic acid, which is a specially designed gene, or its fragments or derivatives thereof, corresponding to a TCR V gene family of human T-cell receptors. Preferred genes, or fragments or derivatives thereof, are those that correspond to the 29 Vα gene or 25 Vβ gene families of human T-cell receptors. (Wilson, *et al., Immunol.* Rev. 101:149 (1988); Roman-Roman, *Eur J. Immunol.*, 21:927(1991); Ferradini, *Eur J. Immunol.*, 21:927 (1991)). The gene array can detect Vα genes or Vβ genes. The gene array can also detect both Vα and Vβ genes by immobilizing both Vα and Vβ gene fragments on the substrate.

The genes, or fragments or derivative thereof, used for the array can be prepared by any conventional method.

One embodiment of the invention is to prepare the genes, or fragments or derivative thereof, by PCR. Recombinant DNA vectors that express DNA fragments of TCRBV, TCRBV and TCRBC and beta-actin genes are prepared by cloning the fragments into pCR2.1 plasmid vector (Ko, *et al., Am. J. Hematol.*, 57:124-130 (1998)); Okeke, *et al., J. Clin. Microbiol.*, 39:3491-4 (2001); Davis, *et al., Clin. Immunol. Immunopathol.*, 89:35-43 (1998)). TCR gene fragments can be amplified, for example, using subfamily-specific oligonucleotide primers (Vα 1 – w29/Vβ 1-w24) by PCR according to Genevee, *et al. (Eur. J. Immunol.* 22:1261-1269 (1992)). Table 1 shows another example of primers for PCR amplification of 25 TCRBV genes (SEQ ID NOs: 1-50), TCRBC gene (SEQ ID NOs: 51 and 52) and beta-actin gene (SEQ ID NOs: 53 and 54). These primers are designed from the public domain of TCRBV and TCRBC. V gene families have large sequence homology. Each set of the primers (SEQ ID NOs: 1-50) is carefully designed such that it specifically represents a particular V gene. Each set of primers is used to amplify each of TCRBV genes, TCRBC gene and beta-actin gene with Taq DNA polymerase by PCR. Each PCR product is then denatured to single-stranded DNAs and immobilized onto a defined position of the substrate.

Table 1: Primers for 25 TCRBV genes, TCRBC and beta-actin genes

GENE #	GENE SEQUENCE 5' → 3'	AMPLICON (bp)
BV1	AAGCACCTGATCACAGCAACT (forward) TAGTTCAGAGTGCAAGTCAGG (reverse)	209
BV2	GGTTATCTGTAAGAGTGGAACCT AGGATGGGCACTGGTCACTGT	229
BV3	TCGAGATATCTAGTCAAAAGGACG GGTGCTGGCGGACTCCAGAAT	228
BV4	AAGCAGGGATATCTGTCAACGT TTCAGGGCTCATGTTGCTCAC	235
BV5	GATCAAAACGAGAGGACAGCA AGCACCAAGGCGCTCACATTCA	217
BV6	CTCAGGTGTGATCCAATTTCA CCCCCGCTCTGTGCGCTGGAT	195
BV7	CATGGGAATGACAAATAAGAAGTCT TGGCTGCAGGGCGTGTAGGTG	214
BV8	CCCCGCCATGAGGTGACAGAG GAGTCCCTGGGTTCTGAGGGC	239
BV9	CCAAAATACCTGGTCACACAG CCAGGGAATTGATGTGAAGATT	207
BV10	ACCTAGACTTCTGGTCAAAGCA GGACTGGATCTCCAAGGTACA	223
BV11	TTATAGGGACAGGAAAGAAGATC ATGTGAGGGCCTGGCAGACTC	224
BV12	CAAGACACAAGATCACAGAGACA GGCAGCAGACTCCAGAGTGAG	224
BV13	TGAAGACAGGACAGAGCATGACA CACAGATGTCTGGGAGGGAGC	227
BV14	ACCCAAGATACCTCATCACAGTG AGAGGTCTGGTTGGGGCTGGG	242
BV15	TCACAAAGACAGGAAAGAGGATT GGGGATGGCAGACTCTAGGGA	215
BV16	GTTCCCCAGCCACAGCGTAATA CAGTTCTGCAGGCTGCACCTT	235
BV17	GTCCCCAAAGTACCTGTTCAGA AGCTGTCGGGTTCTTTTGGGC	244
BV18	AGACACCTGGTCAGGAGGAGG TGCCGAATCTCCTCGCACTAC	240
BV19	CCAGGACATTTGGTCAAAGGAAAA CAGTGCCGTGTCTCCCGGTTC	246
BV20	GACCCTGGTGCAGCCTGTG GAGGAGGAGCTTCTTAGAACT	223
BV21	CCCAGATATAAGATTACAGAGAAA CTGGATCTTGAGAGTGGAGTC	219
BV22	CACAGATGGGACAGGAAGTGATC GTCCTCCAGCTTTGTGGACCG	221
BV23	AAGAGGGAAACAGCCACTCTG CAGCTCCAAGGAGCTCATGTT	207
BV24	CCAAGATACCAGGTTACCCAGTTT CAGGCCTGGTGAGCGGATGTC	228

BV25	AAAACATCTTGTCAGAGGGGAA TGAATCCTCAAGCTTCGTAGC	238
TCRBC	CCGAGGTCGCTGTGTTTGAGCCAT GAGAACTGGTACCGGTAG	496
Beta-actin	AAGTACTCCGTGTGGATCGG AAAGCCATGCCACTCATC	206

TCRBV: T cell receptor beta chain variable region, TCRBC: T cell receptor beta chain constant region.

Preparation of the Array Substrate

TCR gene region arrays are useful research and diagnostic tools for measuring pathogenic T-cell clonal expansion and TCR gene distribution. Each TCR array has a substrate with a plurality of defined positions. A substrate is a supporting material, on which, various genes, or fragments or derivatives thereof, each associated with a particular TCR gene family, are immobilized individually on the defined positions using conventional methods. In addition, each array optionally contains negative controls such as pUC6DNA and pUC18 DNA blanks and house keeping genes (e.g. β-actin, GAPDH, clophilin and ribosomal protein L13a, etc.). Besides acting as positive controls, these house-keeping genes can be used to normalize the signals among arrays, therefore, signals on different arrays can be compared. TCR gene arrays can be low-density or high-density setting. Preferably, the array has a low-density DNA setting to increase the sensitivity of the assay. The low-density DNA setting provides a simple assay to detect and interpret the results. The array substrate can be any solid materials that can immobilize nucleic acids, including, but not limited to membrane and glass. Common membrane includes nylon, nitrocellulose, etc. The gene-array system can be prepared in batches for immediate use or for future use.

With the low density TCR gene array, genes associated with TCR gene such as 25 Vβ gene families and 29 Vα are carefully selected to provide increased sensitivity of detection. A set of 25 primers for analyzing TCR Vβ gene has been illustrated in Table 1.

Sample Preparation

Total RNAs, mRNAs or purified ribosomal mRNAs of T-cells are extracted from a sample, such as a body fluid (e.g. whole blood , serum, plasma etc.) or a cell culture, by a conventional method or commercially available kits. In one embodiment, T-cells are rinsed quickly in ice-cold PBS and RNA is isolated by using TRIzol Reagent (Life Technologies, Rockville, MD) according to the manufacturer's instructions. The RNA quality is insured by gel visualization and spectrophotometric analysis ($OD_{260/280}$). The RNAs are then converted

to signal-labelled cDNA probes by reverse transcription with dNTP mixed with signal agents such as biotin, or fluorescent, chemiluminescent, or radioactive (^{32}P) labelling agents.

Microarray Hybridization and Detection

The labelled cDNA probes are hybridized to TCR gene-specific fragments immobilized on the array under conditions suitable for annealing complementary nucleic acid strands. The array is then washed to remove any unhybridized nucleic acids. The intensity of the hybridization signals is captured by autoradiography for radioactive isotope or by other conventional methods for chemiluminescent, fluorescent, or colorimetric agents, and further analyzed quantitatively by a detector such as a densitometer.

Research and Diagnostic Tools

The present invention is useful in detecting over-expression of certain T-cell receptor V genes in a patient. The sample used can be blood (plasma, serum), tissue (such as synovial tissue) or any body fluid (such as synovial fluid), or bone marrow, derived from the patient. One embodiment of the invention is to detect autoimmune diseases, for example, multiple sclerosis, rheumatoid arthritis, insulin-dependent diabetes mellitus (Falta, *et. al. Clin. Immunol.*, 90:340 (1999)), type I diabetes (Naserke, *et al., Immunogenetics*, 45:87 (1996)), inflammatory bowel disease (Saubermann, *et al., Am. J. Physiol.*, 276:G163 (1999)), psoriasis (Prinz, *et al., Eur. J. Immunol.*, 29:3360 (1999)), system lupus erythematosus (Masuko-Hongo, *et al., J. Clin. Lab. Anal.*, 12:162 (1998)), and Crohn's disease (Ogawa, *et. al., Biochem. Biophys. Res. Commun.*, 240:545 (1997)), which have certain T-cell receptor V genes elevated. Another embodiment of the invention is to detect T cell associated malignancies, for example, T cell leukaemia or T cell lymphoma, which have certain T-cell receptor V genes elevated.

Both rheumatoid arthritis and multiple sclerosis are T cell mediated autoimmune diseases. Previous studies have demonstrated the T cell clonal expansion of specific TCR V genes among these patients. The present invention provides superior research and diagnosis tool to detect and monitor patients with rheumatoid arthritis and multiple sclerosis.

Rheumatoid arthritis is a disease affecting the synovial membrane of the joints, which is thought to result from T-cell-mediated autoimmune phenomena. As an example, activated T cell populations in the synovial tissue of rheumatoid arthritis patients can be examined by analyzing TCR mRNAs isolated from IL2 receptor positive (IL-2R+) synovial T cells. The clonal activation and expansion of Vβ3, Vβ14 and Vβ17 T cells were detected in the synovium

of rheumatoid arthritis patients (Howell, *et al.*, *Proc. Natl. Acd. Sci.*, 88:10921-10925 (1991)); the presence of these T cells indicates rheumatoid arthritis.

Multiple sclerosis is an autoimmune disease mediated by T cells specific for myelin basic protein. Wucherpfennig, *et al.*, *Science*, 248:1016-1019, has applied the PCR to analyze the V region of TCR β chain among 83 T cell lines from both MS patients and healthy subjects that were reactive with the immunodominant region of human MBP (residues 84-102 or 143-168). The study identifies two highly expressed and activated regions of Vβ17 and Vβ12 which were in recognition of MBP.

Using the gene array of the present invention, which has immobilized the complementary sequence of specific TCR V gene sequences, provides an effective method to detect and monitor the disease of rheumatoid arthritis patients and multiple sclerosis with TCR V gene expansion on certain Vβ genes.

The present invention is also useful for detection of clonal T cell proliferations in patients with leukemia and lymphoma. Evaluation of abnormal both B and T cell clonality is important for the diagnosis of lymphoid neoplasms. Previously, McCarthy *et al.* (*American Journal of Pathology* 138: 821-828) has reported the analysis of patients with lymphoid disorders. A series of T cell proliferations in peripheral blood, bone marrow, or tissue samples were analyzed for clonality by using traditional PCR technique to amplify portions of the rearranged TCR beta chain genes; in which both beta-chain alleles were detected to be rearranged.

The present invention is also useful for the analysis and monitoring of the T cell repertoires in clinical situations such as bone marrow transplantation. The analysis of the T cell repertoires involved in local or systemic immune response is important in many clinical situations. These include autoimmunity, response to viral or bacterial superantigens, autoimmunity including autograft rejection, and tumor immunity. Gorski, *et al.*(*J Immunol*, 152:5109-5119 (1994)) used traditional PCR to analyze the complexity and stability of circulating T cell repertoires in adults with bone marrow transplantation. Gorski et al. has found that the repertoire complexity of marrow recipients correlates with their state of immune function. The gene based TCR array provides an effective diagnostic tool to monitor the T cell repertoires among bone marrow transplant donor and recipients.

Another embodiment of the present invention is a ready-to-use assay kit that is prepared based on the above-discussed TCR gene region array system. The kit contains

membranes or other suitable substrate immobilized with DNA encoding specific portions of various T-cell receptor V gene families, along with internal controls (house-keeping genes) for the purpose of quantification. The kit can detect Vα genes or Vβ genes. The kit can also detect both Vα and Vβ genes by using the gene array that has both Vα and Vβ gene fragments immobilized on the substrate. The kit optionally contains solutions required for the assay. Patient specimens are used to prepare mRNA and subsequently hybridized with the substrate. Such a kit can rapidly detect TCR V gene distribution and further identifies T-cell clonal expansion with high accuracy, specificity and sensitivity. The kit is useful in research and clinical laboratories for detection of pathogenic T-cells in various human autoimmune diseases and other pathological conditions. The ready-to-use kits can be manufactured in large quantity.

Further aspect of the present invention is the gene array comprising gene fragments or derivatives thereof corresponding to 29 Vα gene and 25 Vβ gene families of human T-cell receptor immobilized onto a substrate.

The invention is illustrated further by the following examples which are not to be construed as limiting the scope of the specific procedures describing them.

EXAMPLES

Example 1. Protocols of Gene Based TCR Array

Nylon membrane preparation

PCR products preparation

*Plasmid DNA (1ng/μl)	2.0 μl
Specific forward primer (one of BV1-25, or BC)	0.5 μl
Specific reverse primer (one of BV1-25, or BC)	0.5 μl
10mM dNTPs	1.0 μl
10 x reaction buffer (Invitrogen)	5.0 μl
50mM $MgCl_2$ (Invitrogen)	1.5 μl
5 U/μl Taq DNA polymerase (Invitrogen)	0.25 μl
ddH_2O	39.25 μl
Total volume	50.00 μl

* Plasmid DNA is DNA fragment of one of TCBRV 1-25, TCRBC or beta-actin gene, expressed by a recombinant DNA vector.

PCR reactions parameters (optimized conditions)

pre-denaturation	95°C x 3 min	
denaturation	94°C x 30 sec	
annealing	57°C x 30 sec	40 cycles
extension	72°C x 30 sec	
extension	72°C x 5 min	

DNA Spotting to the nylon membrane

1. TCR PCR products (10 µl per nylon membrane) was denatured at 100°C for 5min.; then quickly placed on ice for at least 3 min. Each specific TCR PCR product was transferred into 90 µl 2 x SSC.
2. Nylon membrane was wet in 2 x SSC for 5 minutes and placed onto the blotting device (Bio-dot™ Apparatus, Bio-rad laboratories).
3. Vacuum was pumped at 3 inch Hg. 100 µl DNA solution was spotted into each well. After finishing all TCRBV members and controls, the membrane was put between two 3M filter and baked at 80°C for 3 hours. Dry membranes were saved at room temperature for future usages.

Figure 1 depicts Format of the array membrane design. Each defined position is immobilized with a specific gene format of BV1-BV24, β-actin or pCR 2.1.

Probe labeling and hybridization

^{32}P- cDNA probe synthesis

1. For each total RNA sample, the following was combined into a sterile tube:

Total RNA	*n* µl (< 5µg)
• Cb515 primer (10 pmol/µl)	1.0 µl
*dNTPmix	1.0 µl
RNase-free H_2O	to 12.5 µl

- Cb515 is a primer of TCBRC for cDNA preparation based on reverse transcription.

* dNTPmix is composed of 10mM dATP, dGTP, dTTP, 1mM dCTP and 10 µCi /µl $[\alpha]^{32}$P-dCTP, incubate sample at 65°C for 5 min, then quickly place on ice.

2. Each component was added in the following order.

5 x RT buffer	4.0 µl
0.1 M DTT	2.0 µl
RNase Inhibitor (10 U/µl)	1.0 µl

Sample was incubated at 42 °C for 2 min.

3. 0.5 µl (200 U/µl) Superscript RNase H$^-$ reverse transcriptase (Invitrogen) was added to each sample, mixed and incubated at 42 °C for 25 min. The reaction was terminated at 70 °C for 15 min.

4. The cDNA probe was denatured by heating at 95 °C for 5 min, and chilled quickly on ice for at least 2 min.

Array hybridization and detection

1. 5 ml hybridization solution (6 x SSC, 5 x Denhardt's, 0.5% SDS) was pre-warmed to 60°C for each sample.

2. Sheared salmon sperm DNA (100 µg/ml) was heat-denatured at 95 °C for 5 min, and chilled quickly on ice for at least 3 min. The heat-denatured sperm DNA was added to the pre-warmed hybridization solution to a final concentration of 100 µg DNA /ml, and kept at 60°C until use.

3. The TCRBV array nylon membrane was wetted by adding 3 ml de-ionized water to the hybridization tube containing the array. After the membrane was completely wet, poured off the de-ionized water.

4. 3 ml hybridization solution was added to the hybridization tube. The remaining 2 ml hybridization solution was kept at 60°C until use.

5. The hybridization tube was placed into a hybridization cylinder. TCRBV array membrane was pre-hybridized at 60°C for 1 to 2 hours with continuous agitation at 5-10 rpm/min.

6. Pre-hybridization solution was drained and discarded.

7. The denatured cDNA probe was pre-mixed with the remaining 2 ml hybridization solution and hybridized overnight with continuous agitation at 60°C.

8. The membrane was washed twice by adding 5 ml of pre-warmed wash solution (1 x SSC, 0.1% SDS) to the hybridization tube and incubated in a hybridization oven for 15 min each at 60°C with agitation at 30-40 rpm/min.

9. The membrane was washed twice by adding 5 ml of pre-warmed wash solution (0.1 x SSC, 0.1% SDS) to the hybridization tube and incubated in a hybridization oven for 15 min each at 60°C with agitation at 30-40 rpm/min. The membrane was taken out and wrapped with wrapfilm. The membrane was exposed against X-ray film at –80°C for 6-24 hours to develop the film.

Example 2. Detection of TCRBV Genes of SEBs

This example was designed to study the TCR gene base array. The peripheral blood lymphocyte (PBL) was first activated by staphylococcal enterotoxin (SEB), where SEB is superantigen which stimulates massive T cell proliferation. After the stimulation of PBL with SEB for 48 hours, the TCRBV gene w3as measured by the prepared TCRBV membrane.

As described in Example 1, the nylon membrane was spotted with PCR products specific for TCBRV1-25, TCRBC, beta-actin and pCR2.1 vector. The total RNAs of each sample were isolated and converted to cDNA probe by reverse transcription where Cb515 was used as primer in this step and the radiolabelled nucleotide template mixture was applied. After this process, the radiolabelled probe (sample) was hybridized with the TCBRV array membrane to detect the presence of the TCRBV gene in the sample. Figure 2 demonstrates the detection of TCR BV genes of SEB stimulated normal PBLs.

The invention, and the manner and process of making and using it, are now described in such full, clear, concise and exact terms as to enable any person skilled in the art to which it pertains, to make and use the same. It is to be understood that the foregoing describes preferred embodiments of the present invention and that modifications may be made therein without departing from the scope of the present invention as set forth in the claims. To particularly point out and distinctly claim the subject matter regarded as invention, the following claims conclude this specification.

WHAT IS CLAIMED IS:

1. A method of detecting over-expression of certain T-cell receptor V genes in a sample comprising:

providing a T-cell receptor gene array containing a substrate with a plurality of positions, each position having an immobilized nucleic acid complementary to a fragment of various families of the human T-cell receptor V genes,

extracting RNAs from a sample,

preparing labeled cDNAs from the RNAs by reverse transcription,

contacting said labeled cDNAs with said array under conditions that allow complementary sequences to hybridize;

removing unhybridized nucleic acids; and

identifying one or more positions that have elevated signals compared with other position; whereby the over-expressed T-cell receptor V genes are detected.

2. The method according to Claim 1, wherein said V genes are Vα genes, Vβ genes or the combination of both.

3. The method according to Claim 2, wherein said various families of the human T-cell receptor Vβ genes are selected from the group consisting of Vβ1, Vβ2, Vβ3, Vβ4, Vβ5, Vβ6, Vβ7, Vβ8, Vβ9, Vβ10, Vβ11, Vβ12, Vβ13, Vβ14, Vβ15, Vβ16, Vβ17, Vβ18, Vβ19, Vβ20, Vβ21, Vβ22, Vβ23, Vβ24 and Vβ25.

4. The method according to Claim 2, wherein said various families of the human T-cell receptor Vα genes are selected from the group consisting of Vα1, Vα2, Vα3, Vα4, Vα5, Vα6, Vα7, Vα8, Vα9, Vα10, Vα11, Vα12, Vα13, Vα14, Vα15, Vα16, Vα17, Vα18, Vα19, Vα20, Vα21, Vα22, Vα23, Vα24, Vα25, Vα26, Vα27, Vα28, and Vα29.

5. The method according to Claim 1, wherein the said method detects autoimmune diseases or T cell associated malignancies.

6. The method according to Claim 5, wherein said autoimmune diseases are multiple sclerosis, rheumatoid arthritis, insulin-dependent diabetes mellitus, type I diabetes, inflammatory bowel disease, psoriasis, system lupus erythamatosus, or Crohn's disease.

7. The method according to Claim 5, wherein said T cell associated malignancies are T cell leukemia or T cell lymphoma.

8. The method according to Claim 3, wherein said immobilized nucleic acid is prepared by polymerase chain reaction using primers selected from the group consisting of SEQ ID: NOs. 1-50.

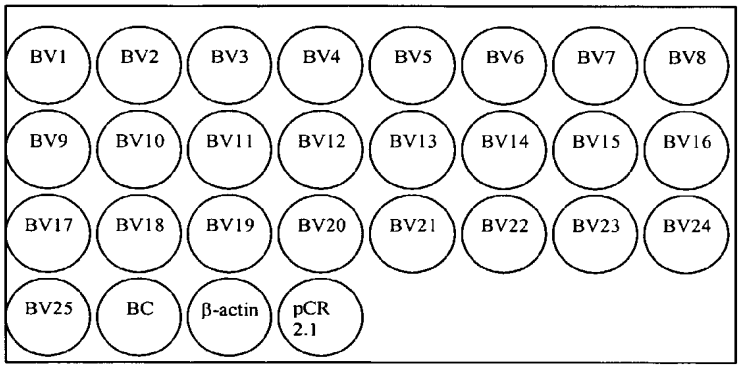
9. A kit for detecting over-expression of certain T-cell receptor V genes in a sample comprising a T-cell receptor gene array, said array containing a substrate comprising a plurality of positions, each position having an immobilized nucleic acid complementary to a fragment of various families of the human T-cell receptor V genes.

10. The kit according to Claim 9, wherein said substrate further comprising additional positions each having an immobilized nucleic acid complementary to a gene that is constitutively expressed in a normal human T-cell.

11. The kit according to Claim 9, wherein said kit detects over-expression of Vα genes, Vβ genes, or the combination of both.

12. The kit according to Claim 9, wherein the substrate comprises immobilized nucleic acids complementary to Vα, Vβ genes, or the combination of both

13. The kit according to Claim 10, wherein said substrate is nylon, nitrocellulose or glass.


BV1
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BV3
BV4
BV5
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BV7
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BV9
BV10
BV11
BV12
BV13
BV14
BV15
BV16
BV17
BV18
BV19
BV20
BV21
BV22
BV23
BV24
BV25
BC
β-actin
pCR 2.1

Figure 1

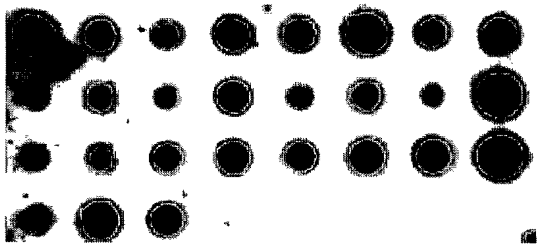

Figure 2